The strain of virus used was obtained from Dr. WERNER HENLE of the Children's Hospital, Philadelphia, and has had 15 mouse intracerebral passages in this laboratory. Roller tube cultures were prepared with minced testes from 5–12-week old Swiss white mice freshly killed with ether, by following essentially the methods of CARREL[1] and GEY[2]. Two types of culture media were employed: (a) 0·5% lactalbumin hydrolysate and (b) a medium consisting of 10% chick embryo juice (1:1 minced chick embryo and buffered HANKS' solution), 10% inactivated horse serum, 20% bovine serum ultrafiltrate, and 60% buffered HANKS' solution. To each were added 100 units penicillin and 100 μg streptomycin per milliliter medium. The cultures were incubated on roller machine (15 revolutions per hour) at 37°C, and confirmed to show good growth of fibroblasts before inoculation with virus.

An initial set of 3 culture tubes, designated T-1, was inoculated with 0·1 ml of 10^{-5} dilution of 10% brain emulsion from mice typically infected with MM virus by an intracerebral route. After 4 days fluids from the tubes were pooled and 0·1 ml of 10^{-1} of this fluid was inoculated into each tube of a second set of the cultures. Successive transmissions in this manner were carried out through the 10th tissue passage (T-10). Amount of virus in each inoculum was determined by intracerebral inoculation of 0·03 ml of ten-fold serial dilutions of the virus-containing fluid into 3-week old Swiss white mice. The 50% lethal doses were calculated by the method of REED and MUENCH[3]. At the 11th tissue culture passage, the growth pattern of the virus was investigated (Fig.1). Samples of fluid in the virus-infected tissue cultures were taken out at 1 h, 12 h, 24 h, 4 days, and 7 days respectively after virus inoculation, and titrated into Swiss white mice for viral activities. All Infected cultures were checked daily for characteristic cytonecrosis[4] by a microscope with 3×, 10×, and 43×, objectives, and a 10× eye piece.

As control, similar examinations were made with uninfected tissue cultures. All fluids harvested were confirmed to contain no bacteria by culturing on tryptone-soybean-yeast extract agar slants for 7 days at 37°C.

In order to ensure that MM virus was actually transferred but not another virus' neutralization tests with immune rabbit serum were performed.

In this study a calculated final dilution of $4{\cdot}89 \times 10^{-26}$ of the original infected mouse brain was obtained at T-11. Virus containing fluid from the eleventh serial tissue passage was capable of initiating infection in Swiss white mice to give characteristic symptoms of paralysis and death and an LD_{50} of $10^{-4{\cdot}6}$. The maximum degeneration of infected fibroblasts was at 5 days after virus infection. All mice inoculated with fluids from uninfected cultures (control cultures) survived.

The findings in this study compare favorably with the results obtained by SMITH and EVANS[4] in their study on the propagation of MM virus in monkey testicular tissue in roller tube cultures, but seem better than the results obtained by CHAMBERS *et al.*[5], who used flask cultures with mouse testicular tissue. Because of the easiness of getting the material as well as the capacity to support viral propagation as evidenced above, the roller tube culture method of mouse testicular tissue is very useful for the study of MM virus. These findings emphasize moreover that this neurotropic virus propagates readily in extraneural tissue.

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Zusammenfassung

11 Passagen von MM-Virus der EMC-Gruppe in Mäusetestikel-Gewebekultur werden beschrieben. Maximale Virustiter von etwa 10^{-4} wurden erreicht; maximale Degeneration der infizierten Fibroblastenkulturen wurde nach 5 Tagen Inkubation beobachtet.

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The Prevention of Experimentally Induced Reticulo-Sarcoma by Hypothyroidism

The injection of trypan blue into rats promotes a high incidence of reticulo-sarcoma involving the liver[1], lymph nodes[2], kidneys and other organs[3]. In a large group of animals some 15 to 18% failed to respond to trypan blue. The reason for this refractoriness has been the subject of our enquiry.

Hypothyroidism[4] as well as inanition[5] can retard the emergence of hepatocellular carcinoma in rats fed butter yellow and can also prevent the development of pituitary tumours in rats in response to chronic oestrogen stimulation[6]. We have also demonstrated that severe malnutrition can almost completely suppress the reticulosis induced by trypan blue, an observation which offers a possible explanation for the decreased incidence of human reticulosis in France reported by CAZAL[7] during World War II. In view of the fact that inanition depresses basal metabolism, the possibility thus presented itself that hypothyroidism would also prevent the emergence of the reticulo-sarcoma induced by trypan blue.

Experimental. 121 adult rats weighing between 250 and 300 g were injected subcutaneously at fortnightly intervals with 1 cm³ of 1% of an aqueous solution of trypan blue (Grubler). Twenty of these rats were fed

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anti-thyroid drugs (thiourea) at a 0·5% level in the diet. The drug was fed first at a level of 0·01% and gradually increased to the 0·5% level. This latter procedure prevented the acute pulmonary oedema known to occur especially after feeding thiourea. The diet was otherwise the same for these experimental rats as for the control series. The remaining 101 rats, constituting the control series, were treated with trypan blue only and were not fed any of the antithyroid drugs. Since 3 of the rats fed thiourea and 5 of the control rats died before the 100th day of the experiment, only 17 of the experimental rats and 96 controls could be reported as the effective number to be used for comparison.

71 of 96 control rats, that is 76% developed reticulosarcoma whereas not a single experimental rat developed such a tumour. These results are statistically significant.

Examination of the livers of the trypan blue-treated rats fed antithyroid drugs revealed that whereas tumour formation was inhibited, only in one instance was the trypan blue reaction completely suppressed. In the remaining 16 livers, there was mild portal reticulosis, and the appearance of fluid-filled cysts, similar to those described previously[1]. However, with one exception, the trypan blue response in the rats fed antithyroid drugs at the end of 200 to 400 days was not greater than that observed in control animals at the end of the 40th day of the experiment. In the exceptional instance, the reaction at the 140th day was comparable with that occurring in the control rats at about the 70th day of treatment.

From the foregoing, it can be concluded that a measure of thyroid activity is essential for the development of reticulo-sarcoma in response to trypan blue. Depression of thyroid function below a defined level, whether achieved by antithyroid drugs or by underfeeding, will not only depress the intensity of the reticulosis but indeed excludes the development of reticulo-sarcoma. Depression of the thyroid therefore retards and can even prevent the emergence of experimentally induced reticulo-sarcoma as well as carcinoma of the liver and of adenoma of the pituitary gland.

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Zusammenfassung

Die Entstehung von Reticulosarkom bei Ratten nach mehrmaliger Injektion von Trypanblau kann unterdrückt werden durch Entkräftung, Verabreichung antithyreoidaler Mittel und durch alle andern Faktoren, welche den Stoffwechsel herabsetzen.

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The „Feeding Centre" of the Hypothalamic Region of the Rat Brain

According to BROBECK *et al.*[1], the sense of hunger and the food intake is regulated through a "feeding centre" localized in the lateral parts of the anterior hypothalamus. An attempt to analyse the biochemical mechanism of the regulation was made, applying the tracer technique. Groups of hungry (H) rats were administered 200 μC $Na_2HP^{32}O_4$ intraperitoneally at the end of a 24 h hunger-period, together with fed (F) ones. They were sacrificed 15–60 min later through plunging in liquid oxygen. In other sets of experiments the isotope was given at the start of the hunger period and the animals were sacrificed at the end. Besides the feeding centre, C, two adjacent parts of the hypothalamus, denoted A and B, were prepared from the frozen brain tissue, likewise samples of cerebrum, blood, liver and muscles were taken for comparison of the gross distribution of activity in H and F animals.

Pooled brain samples of H and F rats were analyzed with regard to:

(a) the distribution of P^{32} per unit volume of the samples;
(b) the partition of P^{32} on the trichloroacetic acid (TCA) fraction, the lipid fraction and the combined protein + nucleic acid fraction;
(c) the partition of P^{32} within the TCA-fraction[1].

Determinations of total phosphorus in the A–C samples and of orthophosphate and the hydrolysable end-groups of ATP+creatine phosphate, as well as of total creatine[2] (free creatine+creatine phosphate) in the TCA fractions, were also performed.

The liver of H rats took up appreciably more ^{32}P than the liver of F animals, whereas the concentration in the muscles, the cerebrum and the blood was similar within experimental errors. This suggests that the distribution of ^{32}P through the blood to other parts of the brain than the hypothalamus does not differ in the two groups of animals (Table I).

The distribution of P^{32} on the hypothalamic samples under consideration shows a characteristic pattern (Table I) which may be described in the following way: while the *C* samples of H rats accumulate more activity than those of F rats, the opposite is true for samples *A* and *B*. This is simply demonstrated through the ratio's *C/A* and *C/B* which measures are statistically different in H and F rats. No significant differences occured in the amount of total phosphorus which account for these changes. The same general mode of distribution was found when the samples were chemically fractionated according to (b) and the activity of the single fractions determined. The analyses thus suggest an all-over change in the biochemical activity of the samples of hungry rats as compared with fed ones.

It is of interest to note that the integrated activity in the three samples $A + B + C$, making up about $^2/_3$ of the total hypothalamus, does not differ appreciably in the two groups of rats. This indicates that the P^{32} supply through the blood to the hypothalamic region as a whole is rather unimpaired by the state of hunger. Obviously, then, the partition of activity within the hypothalamus is autonomously regulated.

In further analyses, the concentration of the hydrolysable end-groups of ATP+creatine phosphate was found mainly to follow the same pattern of distribution as the P^{32} given in Table I, while the concentration of orthophosphate seems to vary less (Table II). Likewise the distribution of total creatine agreed with the general scheme. Calculations of the amount of activity present

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